

## LEUCOCYTIC REACTION TO BACTERIAL INFECTION IN ANIMALS\*

SISTER M. FRANCIS XAVIER O'REILLY

CERTAIN changes have been noted in the circulating polymorphonuclear leucocytes in men and the lower mammals during infections associated with the production of pus. Summarized briefly they are: (1) increased basophilia (acid reaction) of the cytoplasmic granules; (2) pycnosis of the nucleus (deep staining and clumping of the chromatin); (3) formation of basophilic nongranular areas (Döhle bodies) in the cytoplasm; and (4) vacuolization of the cytoplasm. When the bone marrow is unable to supply mature polymorphonuclear leucocytes in adequate numbers immature forms appear (the so-called "shift to the left").

In a series of experiments (O'Reilly, 1936) in which large doses of *Staphylococcus aureus* were injected into the rat an infectious state was produced and was accompanied by basophilia of the granules of the neutrophils, similar to that which is found in man during the course of a pyogenic infection.

To analyze the process of leucocytic behavior and immunity in man certain aspects of it have been studied in lower animals which are normally exposed to infecting media in their environment.

### MATERIAL AND METHODS

In this analysis *S. aureus* (from a case of osteomyelitis) was injected into earthworms, crayfish, yellow perch, goldfish, mud puppies, frogs, garter snakes, turtles, pigeons, guinea pigs, rabbits, and mice. Mice were also inoculated with suspensions of lampblack and fibrin in order to compare the reactions of the leucocytes to an infecting organism and to a particulate matter (lampblack) and a foreign protein (fibrin).

The animals were kept under standard laboratory conditions. Films of blood in circulating body fluids stained by Wright's method

\* Contribution from the Department of Zoölogy and the Thomas Henry Simpson Memorial Institute for Medical Research of the University of Michigan.

were studied before the animals were inoculated and at intervals of twenty-four hours after injection of measured amounts of the infecting organism. The types of leucocytes normally found in each species are listed in Tables I and II.

#### ACKNOWLEDGMENTS

This study was conducted under the direction of Dr. Alvalyn E. Woodward and Dr. Raphael Isaacs, whose guidance has been invaluable. Dr. Malcolm E. Soule provided the infecting organism and Dr. Frank E. Eggleton identified the crayfish. Photomicrographs were taken by Mr. William Cristanelli. I wish to offer my sincere gratitude for and appreciation of this generous assistance.

#### EXPERIMENTAL ANIMALS

##### *Earthworm (Lumbricus terrestris)*

Twenty-four hours after the first injection of staphylococci into the coelom of an earthworm mononucleated cells of the circulating fluid were filled with these organisms (Pl. II, Fig. 1), and twenty-four

### TABLE I

#### CELLS OF THE CIRCULATING FLUID OF TWO GROUPS OF INVERTEBRATES

The classification of the cells of the coelomic fluid of the earthworm was compiled from a comparative study of the normal cells of *Lumbricus terrestris* and from the description of them by Lim Boon Keng (1895); that for the cells of the crayfish was taken directly from Tait and Gunn (1918), after the normal cells and the description of them by Hardy (1892) and Meinertz (1902) had been studied.

##### *Earthworm*

|                                                                             |                                                                                                                                                                     |                                                  |
|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Lymphocytes, spherical cells with deeply basophilic coarse granular nucleus | Monocytes (phagocytic, mononuclear), large hyaline cells; inner zone of cytoplasm contains fine eosinophilic granules; peripheral zone contains basophilic granules | Small spherical cells with eosinophilic granules |
|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|

##### *Crayfish*

|                                                            |                                                                                      |                                                                                               |
|------------------------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Explosive cells, spindle-shaped cells, and central nucleus | Hyaline thigmocytes (phagocytic); cytoplasm contains a large number of fine granules | Granular thigmocytes, variable in form; oval nucleus and cytoplasm filled with large granules |
|------------------------------------------------------------|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|



hours later the last few segments of the tail region were sloughed off spontaneously. Sections of these discarded terminal segments were filled with bacteria, which were distributed in the coelomic fluid, in the phagocytes, and in the chloragogue cells of that region. Each additional injection was followed by further loss of segments. Some of the animals shed as many as four times.

The regularity with which autotomy of the terminal segments containing phagocytes and bacteria followed injection led to the conclusion that in the earthworm foreign particles are ingested by phagocytic cells of the coelomic fluid and are carried to the posterior segments, which are then discarded. No cellular changes, such as digestion of the bacteria within the cell, are necessary to this animal in resisting infection.

#### *Crayfish (Cambarus virilis)*

After one day of infection with *S. aureus* the organisms were found in the hyaline thigmocytes of the circulating body fluid. Although these cells were studied in a variety of preparations, in films dried in air and stained with dilute methylene blue, in other films fixed in osmic acid vapor and stained with Wright's stain, in still other films stained with brilliant cresyl blue (vital stain), and in a hanging drop to which the infecting organism had been added, phagocytosis of the organisms was the only change observed. No evidence of the digestion of the bacteria contained in the cells or of any cell inclusions that resemble the granules of the neutrophils of man was visible. Two days after an injection an almost complete absence of phagocytes in the circulating fluid was noted. From this observation we can assume that the species possesses a peculiar mechanism for ridding itself of infecting organisms.

#### *Yellow Perch (Perca flavescens) and Goldfish (Carassius auratus)*

When staphylococci were first injected into the perch and goldfish the number of leucocytes increased. Continued injections (second, third, and fourth) on successive days maintained this increase in the number of white blood cells, which was also accompanied by phagocytosis (Pl. I, Fig. 2), pycnosis of the nucleus (Pl. I, Fig. 4c), and vacuolization in the cytoplasm. These pathological cells should be compared with the normal neutrophil (Pl. I, Fig. 1). During the course of infection the ingested bacteria underwent a

TABLE II

## LEUCOCYTES OF VARIOUS GROUPS OF VERTEBRATES

The descriptions of the leucocytes are taken from the work of other authors, as follows: for amphibians and mammals, from Scarborough (1930); for perch and goldfish, from Loewenthal (1928, 1930 a and b); for the pigeon, from Kennedy and Climenko (1928). Classification of the leucocytes in the garter snake and the turtle is the result of the author's study of the normal cells and of descriptions of the leucocytes in the literature of the subject.

| Animals infected  | Lymphocyte            | Monocyte, mononuclear                     | Neutrophil                                                                                                              | Eosinophil                                                                         | Basophil                      |
|-------------------|-----------------------|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------|
| Perch . . .       | Large and small forms | .....                                     | Contains fine neutral granules                                                                                          | .....                                                                              | .....                         |
| Goldfish . .      | Normal                | Normal                                    | Nucleus may be simple or polymorph                                                                                      | Large cell with eccentric nucleus                                                  | .....                         |
| Necturus and frog | Normal                | .....                                     | Polymorphonuclear; polynuclear, but may be monolobed or bilobed; contains a few scattered fine metachromatic granules * | Normal                                                                             | Normal                        |
| Snake . . .       | Normal                | Large cell with large eccentric nucleus   | .....                                                                                                                   | Large cell with lens-shaped polar nucleus, spherical or ellipsoidal red granules * | Mononuclear cell              |
| Turtle . . .      | Normal                | Large nongranular cell, spherical or oval | .....                                                                                                                   | Lens-shaped polar nucleus, large red spherical or ellipsoidal granules *           | Large and small varieties     |
| Pigeon . . .      | Small, mononuclear    | Large                                     | .....                                                                                                                   | Bilobed, with spherical or rod-shaped granules *                                   | Spherical or reniform nucleus |

\* Phagocytic.

TABLE II (Concluded)

| Animals infected | Lymphocyte                   | Monocyte, mononuclear             | Neutrophil                                                                        | Eosinophil                  | Basophil          |
|------------------|------------------------------|-----------------------------------|-----------------------------------------------------------------------------------|-----------------------------|-------------------|
| Guinea pig       | Resembles lymphocytes in man | Some cells contain Kurloff bodies | Pseudoeosinophil, 6-8-lobed nucleus, granules round, slightly basophilic *        | Resembles eosinophil in man | Polymorphonuclear |
| Rabbit..         | Resembles lymphocytes in man | Normal                            | Pseudoeosinophil or amphophile, polymorphonuclear, slight affinity for basic dyes | Polymorphonuclear           | Polymorphonuclear |
| Mouse...         | Normal                       | Normal                            | Polymorphonuclear                                                                 | Polymorphonuclear           | Polymorphonuclear |

\* Phagocytic.

gradual change: they increased in size (Pl. I, Fig. 3a), liquefied, completely filled the vacuoles that had formed around them, and became globular masses (Pl. II, Figs. 2-4) in the cytoplasm. As the digested material was absorbed by the cell the globules shrank (Pl. I, Fig. 4b) and changed in staining reaction from a red violet to blue, which gave them the appearance of Döhle bodies (Pl. I, Fig. 5d). The basic-staining areas are not similar to the basophilic granules seen in man during the course of an infectious disease, but they suggest a source of such basophilia.

*Mud Puppy (Necturus maculosus) and Frog (Rana pipiens)*

An increase in the number of leucocytes, phagocytosis, pycnosis of the nucleus, formation of vacuoles in the cytoplasm, and change in the staining reaction from a red violet to a blue were observed in the polymorphonuclear neutrophil (Pl. II, Fig. 6) and in a few mononuclear cells (Pl. II, Fig. 5) as the result of four successive daily injections into the mud puppy and frog. Vacuoles formed around the ingested staphylococci, which liquefied during digestion and created acid areas (Döhle bodies) in the cytoplasm. Nothing ap-



peared to suggest basophilia of the granules of the neutrophils as it appears during a pyogenic infection in man.

*Garter Snake (Thamnophis sirtalis)*

The first injection into the garter snake produced an increase in the number of leucocytes and a slight phagocytosis by the monocytes. Successive inoculations (two and occasionally a third) not only caused an increase of phagocytes, which included mononucleated cells and eosinophils with ellipsoidal granules, but also produced pycnotic nuclei and cytoplasmic vacuoles in the phagocytic cells. Changes in the staining reaction of the ingested bacteria from a red violet to a blue occurred as these organisms underwent digestion.

After the third dose of staphylococci a few inches of the tail of one of the snakes became swollen and discolored. Films made from the blood of this region showed many phagocytes and free bacteria (Pl. III, Fig. 1). The animal recovered without undergoing any further change.

*Turtle (Emys blandingii)*

Four injections administered daily into the coelom of the turtle increased the number of leucocytes and produced vacuoles in the cytoplasm and an almost spherical swelling of the ellipsoidal granules of the eosinophils. Some of these eosinophils were broken while the films were being made, and it was in these cell fragments that staphylococci were observed well embedded near the pycnotic nucleus. Phagocytes were not numerous in the blood films, but there was an increase in the number of the eosinophils with ellipsoidal granules, in the fragments of which were phagocytosed bacteria.

*Pigeon*

The first injection into the pigeon produced a definite leucocytosis which, unlike the condition in other groups, persisted for several days after successive inoculations (five) had been discontinued. In the pigeon the eosinophil with rod-shaped granules is the phagocytic cell, and during the time of infection these granules became so swollen that they gave the cell a "plump" appearance (Pl. III, Fig. 2). The change in shape was accompanied by vacuolization of the cytoplasm and changes in staining reaction from a lighter to a darker blue. The digested material in the swollen granule never quite filled the vacuole as it did in other animals.

*Guinea Pig*

One injection produced an increase in the number of leucocytes. No further change was apparent after a second injection. Basophilia of some of the granules of the pseudoeosinophils (Pl. III, Fig. 3) followed the third injection, and basophilia of a greater number of granules ensued after the fourth. This basophilia of the granules of the pseudoeosinophils of the guinea pig is similar to that of the polymorphonuclear neutrophils appearing in man during the course of an infectious disease.

Two guinea pigs died after receiving extra large doses of unusually virulent *S. aureus*. Blood taken from their hearts showed all the pseudoeosinophils filled with staphylococci.

*Rabbit*

Three subcutaneous injections were given on alternate days to the rabbits. The infection so induced was accompanied by basophilia of the granules of the pseudoeosinophils (Pl. III, Fig. 6), similar to that found in human neutrophils during the course of an infectious disease.

*Mouse (Mus musculus)*

Young mice of C57 (black) and dba (dilute brown) varieties from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, were used in this experiment.

In the normal neutrophils of the black mice no granules occur. After two days of infection a few fine slightly basophilic granules appeared in the cytoplasm of the neutrophils. A third day of infection increased the number of basophilic granules of these cells (Pl. III, Fig. 5). No further change could be caused.

In the dilute brown variety of mice two injections produced basophilia of the granules of some of the neutrophils. Basophilia of the granules of a greater number of the neutrophils followed the third injection (Pl. III, Fig. 4). In both varieties of mice this basophilia was similar to that observed in the rat in a series of experiments by the writer (O'Reilly, 1936) and to that found in man during a bacterial infection.

Two groups of mice were inoculated with a sterile suspension of lampblack in saline: one group received three injections at weekly intervals; the other group, the same number but on successive days.



Twenty-four hours after the first injection deposits of this substance were present in the neutrophils (Pl. I, Fig. 6). Similar deposits were observed several days after the inoculations had been discontinued.

A sterile suspension of powdered fibrin (one per cent in physiological salt solution) was also used to inoculate two groups of mice. Two died a few hours after the first inoculation: one a few hours after the second inoculation of fibrin; the other shortly after a third inoculation of the substance. In the blood films made after the injections only an occasional neutrophil was found. Neither basophilia of the granules nor any change in the cytoplasm of the few neutrophils remaining in the circulation was evident.

#### DISCUSSION

Results of this investigation of the reaction of leucocytes in a variety of animals to the presence of an infecting organism indicate that in each class of animals studied a particular reaction or set of reactions is induced to protect the animal against bacteria and their metabolic products. In man reaction to infection can be noted by visible changes in the neutrophils. Normal neutrophilic granules become more acid (basophilia of the granules), acid areas (Döhle bodies) appear in the cytoplasm, vacuolization occurs, and the nucleus becomes more compact (pycnotic).

In the animals studied many of these changes, such as the presence of vacuoles and acid areas which resemble Döhle bodies in the cytoplasm and pycnosis, were duplicated. In the lower vertebrates these reactions were preceded by phagocytosis. Basophilia of the granules was limited to mammals, whereas in the invertebrates the cells of the circulating body fluids were actively phagocytic, but presented none of the changes noted in the vertebrates. From these observations we may conclude that the visible mechanisms for resisting the action of infecting organisms in the blood or circulating fluid of the groups of animals under investigation fall into three distinct categories: (1) phagocytosis and excretion; (2) phagocytosis and digestion; and (3) basophilia of the granules of the polymorphonuclear neutrophils or their homologues, the pseudoeosinophils.

Cells of the circulating body fluids of the earthworm defend themselves against bacteria by phagocytosis and autotomy. Cuénot (1897) and Willem and Minne (1899; cited by Lison, 1928) acknowl-



edge the existence of an excretory function of the "amoebocytes" of *Lumbricus* and describe the passage of cells containing particles of injected dye from the coelom into the lumen of the intestine, where they are excreted with the feces. Lison (1928), commenting on the method by which the animal rids itself of tubercle bacilli, says that the organisms are removed by phagocytosis and excretion, and Keilin (1925) finds that, when this animal is infected with parasites, it carries the infecting organisms to the posterior end of the body, where the last few parasite-laden segments are removed by autotomy.

The present study likewise ascribes an excretory rôle to the mononucleated cells, since they engulf the staphylococci that have been injected into the coelom and carry them to the posterior end of the body, where the segments containing them drop off.

The hyaline thigmocytes of the crayfish were definitely phagocytic, but there was no evidence of digestion of the engulfed bacteria; nor were there any cell inclusions that resembled in any way the granules of the neutrophils of man. Although it may be assumed from these observations that the organisms were excreted by the hyaline thigmocytes which contained bacteria after injection and which were definitely phagocytic in a hanging drop, yet these phagocytes never appeared in great numbers in the stained films, and, as a rule, had almost entirely disappeared forty-eight hours after injection. Helen Pixell-Goodrich (1929), in describing an infectious disease of *Gammarus*, another crustacean, says it is sometimes cured by phagocytosis and autotomy. Though removal of infecting organisms by autotomy is not proved in this study, some evidence has been accumulated which indicates that it occurs.

A second mechanism, that of engulfing and digesting the invading organism, was observed in the lower vertebrates. Leucocytes of the fish, amphibians, and snakes ingested the organisms, formed vacuoles around them, appeared to pour secretions into the vacuoles which digested the bacteria, and then absorbed the soluble material. As digestion and absorption proceeded, the reaction of the digested material changed, so that definitely acid areas which resembled Döhle bodies appeared in the cytoplasm.

An outstanding difference noted in the leucocytes of the snake and in those of the fish and amphibians studied is the inclusion of a granular cell among the phagocytes of the former. Although granules

were present in these cells, there was no suggestion of basophilia of these granules like that seen in man during pyogenic infection.

The ellipsoidal or rod-shaped granules of the eosinophils of the snake, turtle, and pigeon became swollen and assumed an almost spherical shape after injection. This change followed phagocytosis and was accompanied by vacuolization in the cytoplasm, particularly around the ingested organisms, and by digestion and absorption of the engulfed bacteria. The organisms changed in their staining reaction from a red violet to a blue, except in the pigeon, in which the change was from a lighter to a darker blue. As the pigeons recovered from infection the rod-shaped eosinophilic granules that had become swollen lost their spherical shape and became normal in appearance.

Mammals included in this experiment, that is, guinea pigs, rabbits, and mice, responded to injection by a basophilia of the granules of the neutrophils which was similar to the basophilia found in the human neutrophil during the course of an infectious disease. This group showed no evidence, however, of the phagocytosis engendered among the lower vertebrates.

Infection in man is known to produce changes in the nucleus and cytoplasm, and basophilia of the granules of the neutrophils. These same changes were observed in the phagocytic cell of the leucocytes of the vertebrates investigated in this report, although in each group below the mammals they were accompanied by visible phagocytosis. Furthermore, as the engulfed organisms underwent digestion, changes occurred in the staining reaction of the ingested bacteria in the vertebrates but not in the invertebrates. Thus we may conclude that the infecting organism was removed from the invertebrates studied by means other than digestion.

Early in this investigation it became apparent that, if ten or more days were allowed to elapse between the first and the second inoculations, subsequent doses had no visible effect. During the intervening days the animal built up an immunity against the infecting organism. In order to obtain reactions in which the bacteria or their toxins caused definite changes in the cells it was necessary to repeat the inoculations on at least three successive days. That the changes so induced resulted from the injections of *S. aureus* is obvious since the injections of neither lampblack, a particulate matter, nor fibrin, a foreign protein, effected any discernible alteration in the staining properties of the leucocytes.

During the course of the disease some of the infected animals showed signs of it in their appearance and behavior. This was true especially of the snakes, pigeons, rabbits, and mice. Loss of resistance to handling, a drooping appearance, and refusal to eat were common symptoms of infection among the several groups. These clinical changes suggest that the reactions were caused by infection rather than by the injection of inactive particles.

#### CONCLUSIONS

1. Large nongranular mononucleated cells of the earthworm engulf infecting organisms and carry them to the posterior end of the body, the last few bacteria-laden segments of which are lost by autotomy.

2. Hyaline thigmocytes of the crayfish phagocytose the infecting organisms and then remove them from the circulating body fluid.

3. Phagocytic leucocytes of perch, goldfish, mud puppy, frog, and snake (monocyte) engulf and digest the injected *S. aureus*, after which changes of the nucleus (pycnosis) and the cytoplasm (vacuolization) are observed. Acid areas resembling Döhle bodies appear in the cytoplasm during the absorption of the digested material.

4. Eosinophils with ellipsoidal or rod-shaped granules engulf the *S. aureus* injected into the snake, turtle, and pigeon. Subsequent changes observed are pycnosis in the nucleus and vacuolization in the cytoplasm. In the snake the eosinophils constitute a second form of phagocyte.

5. Basophilia of the granules in the pseudoeosinophils of the guinea pig and the rabbit and in the neutrophils of the mouse appear after three days of infection with *S. aureus*.

MOUNT MERCY COLLEGE  
PITTSBURGH, PENNSYLVANIA

#### LITERATURE CITED

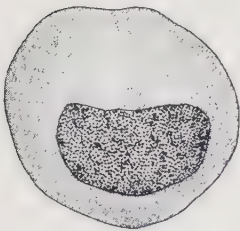
- CUÉNOT, L. 1897. Études physiologiques sur les Oligochètes. Arch. de Biol., 15: 79-124.
- HARDY, W. B. 1892. The Blood Corpuscles of the Crustacea, together with a Suggestion as to the Origin of Crustacean Fibrin-ferment. Journ. Physiol., 13: 165-190.
- KEILIN, D. 1925. Parasitic Autotomy of the Host as a Mode of Liberation of Coelomic Parasites from the Body of the Earthworm. Parasitology, 17: 170-172.



- KENG, LIM BOON. 1895. On the Coelomic Fluid of *Lumbricus terrestris* in Reference to a Protective Mechanism. Trans. Roy. Soc. London, 186 B: 383-401.
- KENNEDY, W. P., AND CLIMENKO, D. R. 1928. Studies on the Blood of Birds. I. The Corpuscles of the Pigeon. Quart. Journ. Physiol., 19: 43-49.
- LISON, L. 1928. Contribution à l'étude des Hémamibocytes des Oligochètes terrioles. Arch. de Biol., 38: 411-455.
- LOEWENTHAL, N. 1928. Étude sur les globules blancs du sang dans le série des vertébrés. Arch. d'anat. d'histol. et d'embryol., 8: 225-306.
- 1930a. Nouvelles observations sur les globules blancs du sang chez les animaux vertébrés. *Ibid.*, 11: 247-272.
- 1930b. De l'évolution des globules blancs du sang dans la série des vertébrés. Comptes Rendus Acad. Sci., Paris, 191: 1072-1074.
- MEINERTZ, J. 1902. Beiträge zur vergleichenden Morphologie der farblosen Blutzellen. Arch. f. path. Anat. . . , 168: 353-398.
- O'REILLY, SISTER M. FRANCIS XAVIER. 1936. Production of the Basophilia of the Granules of the Polymorphonuclear Neutrophilic Leucocytes in Experimental Infection of the Rat. Journ. Lab. and Clin. Med., 21: 1137-1141.
- PIXELL-GOODRICH, HELEN. 1929. Reactions of *Gammarus* to Injury and Disease, with Notes on Some Microsporidial and Fungoid Disease. Quart. Journ. Microsc. Sci., 72: 325-353.
- SCARBOROUGH, R. A. 1930. The Blood Picture in Normal Laboratory Animals. Yale Journ. Biol. Med., 3: 63-80, 169-179.
- TAIT, J., AND GUNN, J. D. 1918. The Blood in *Astacus fluviatilis*: a Study in Crustacean Blood, with Special Reference to Coagulation and Phagocytosis. Quart. Journ. Exper. Physiol., 12: 35-80.
- WILLEM, VICTOR, AND MINNE, ACHILLE. 1899. Recherches sur l'excrétion chez quelques Annélides, Vol. LVIII. Mémoires des savants étrangers, publiés par l'Académie royale des Sciences, des Lettres et des Beaux-arts de Belgique.

## EXPLANATION OF PLATE I

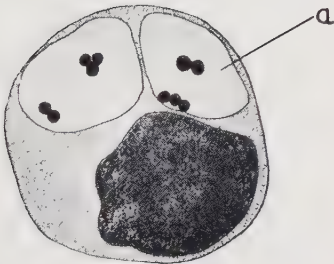
- FIG. 1. Normal neutrophil of perch
- FIG. 2. Neutrophil of perch, showing phagocytosed bacteria and vacuoles in the cytoplasm
- FIG. 3. Neutrophil of perch, showing enlargement of the staphylococci in the vacuole (a)
- FIG. 4. Neutrophil of perch, showing globules in the cytoplasm (b) and pycnosis of the nucleus (c)
- FIG. 5. Neutrophil of perch, showing a few small globules in the cytoplasm resembling Döhle bodies (d)
- FIG. 6. Neutrophil of mouse, showing phagocytosed lampblack



1



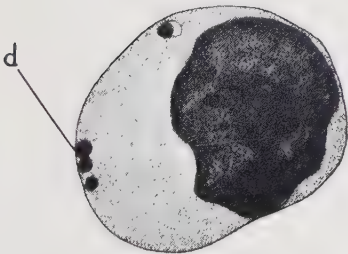
2



3



4



5



6

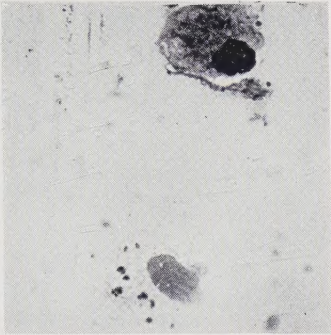
E. Herbold, del.

## EXPLANATION OF PLATE II

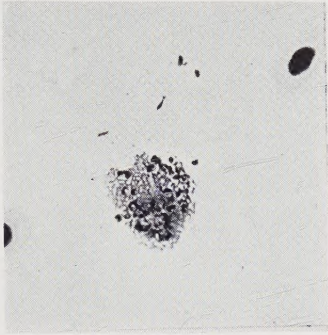
The explanations apply to the white blood cells only.

- FIG. 1. Monocyte of earthworm containing phagocytosed bacteria. Twenty-four hours after first inoculation
- FIG. 2. Neutrophil of goldfish containing phagocytosed bacteria and showing early globule formation. Twenty-four hours after third inoculation
- FIG. 3. Neutrophil of perch containing phagocytosed bacteria and showing early globule formation. Twenty-four hours after third inoculation
- FIG. 4. Neutrophil of perch showing globule formation. Forty-eight hours after fourth inoculation
- FIG. 5. Mononuclear leucocyte of *Necturus* containing phagocytosed bacteria. Twenty-four hours after third inoculation
- FIG. 6. Polymorphonuclear neutrophil of frog containing phagocytosed bacteria. Twenty-four hours after first inoculation

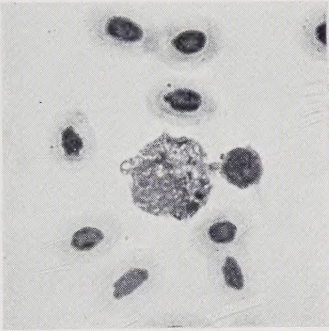




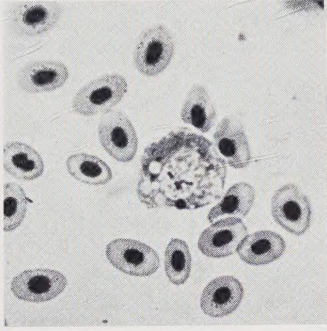
1



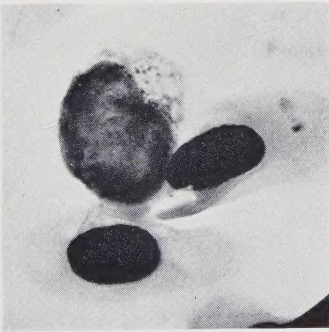
2



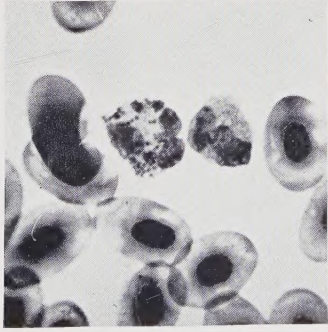
3



4



5

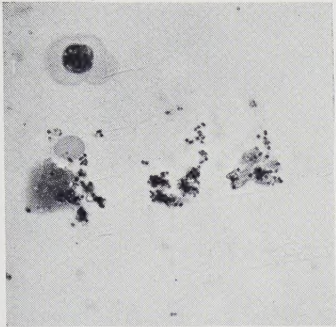


6

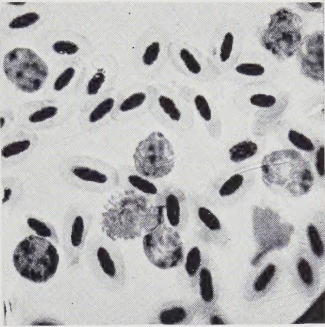
### EXPLANATION OF PLATE III

The explanations apply to the white blood cells only.

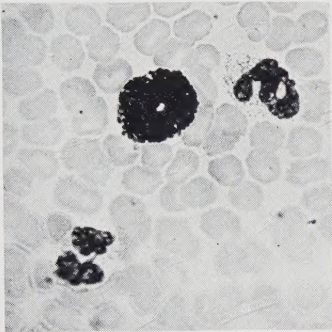
- FIG. 1. Monocytes of snake. Blood taken from swollen tip of the tail after third inoculation. Note the appearance of the leucocytes and the number of bacteria
- FIG. 2. Eosinophils containing rod-shaped granules of pigeon. Forty-eight hours after fourth inoculation
- FIG. 3. Pseudoeosinophil (lower left and upper right) of guinea pig, showing fine basophilic granules. Twenty-four hours after third inoculation
- FIG. 4. Polymorphonuclear neutrophils of brown mouse. Twenty-four hours after third inoculation
- FIG. 5. Polymorphonuclear neutrophils of black mouse. Twenty-four hours after third inoculation
- FIG. 6. Pseudoeosinophils of rabbit showing basophilic granules. Twenty-four hours after third inoculation



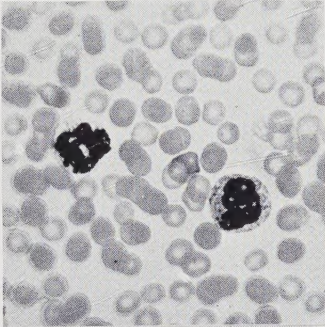
1



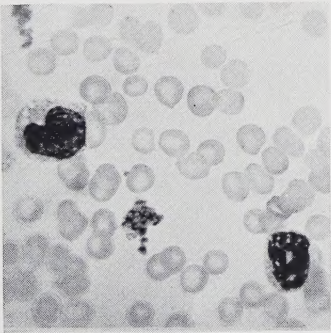
2



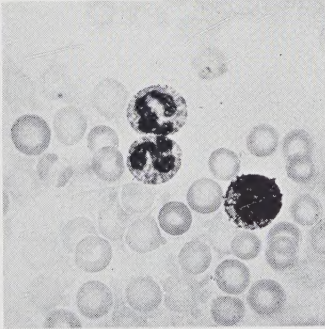
3



4



5



6



